This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A competitive method for estimating the concentration in a sample of a

detecting Bacillus anthracis in a sample comprising the steps:

a. intermixing a said sample, suspected of containing said Bacillus anthracis polypeptide

with a specific antibody to said polypeptide Bacillus anthracis protective antigen, and a

competitive reagent consisting comprising of a Bacillus anthracis polypeptide labeled

with a fluorochrome capable of binding to said specific antibody, to produce a mixture:

b. incubating said mixture for 15 seconds to 5 minutes:

e. detecting binding interaction of said Bacillus anthracis polypeptide in the sample and

antibody by fluorescence polarization, fluorescence lifetime or fluorescence resonance

energy transfer.

c. measuring the fluorescence polarization of said mixture, a negative control solution of

said fluorochrome-labeled competitive reagent, and a positive control solution of said

fluorochrome-labeled competitive reagent exposed to a known amount of said Bacillus

anthracis protective antigen;

d. detecting binding interaction between said Bacillus anthracis protective antigen in the

sample and said antibody by comparing the measured fluorescence polarization of said

mixture with the measured fluorescence polarization of said negative control solution.

and said positive control solution.

2

Attorney Docket No.: NC 84,641 Application Ser. No. 10/809, 877

Claim 2 (canceled).

Claim 3 (withdraw): The method of claim 1, wherein said detection is by change in fluorescence

life-time.

Claim 4 (withdraw): The method of claim 1, wherein said detection is by sensitized fluorescence

of the acceptor or by quenching of donor fluorescence or by fluorescence depolarization.

Claim 5 (canceled)

Claim 6 (currently amended): The method of claim 1, wherein said competitive reagent is native

or recombinant Bacillus anthracis protein or fragments of said protein-polypeptides.

Claim 7 (original): The method of claim 1, wherein said protein can be detected down to less than

5 μg/ml or 6 nM within 5 minutes.

Claim 8 (original): The method of claim 1 wherein said sample is selected from the group

consisting of broth culture media of growing Bacillus anthracis or bodily fluids.

Claim 9 (currently amended): The method of claim 1, wherein said incubation step (b) occurs in

less than 30 seconds for samples suspected of containing high concentration of said Bacillus

anthracis protective antigen.-concentrated samples.

3

Attorney Docket No.: NC 84,641 Application Ser. No. 10/809, 877

Claim 10 (currently amended): The method of claim 1 wherein said incubation step (b) occurs in 4 to 5 minutes for <u>samples suspected of containing</u> low concentration <u>of said Bacillus anthracis</u> protective antigen—samples.

Claim 11 (original): The method of claim 1 wherein said fluorochrome is pH independent.

Claim 12 (original): The method of claim 1 wherein said fluorochrome is selected from the group consisting of 7-AAD, Acridine Orange, Alexa 488, Alexa 532, Alexa 546, Alexa 568, Alexa 594, Aminonapthalene, Benzoxadiazole, BODIPY 493/504, BODIPY 505/515, BODIPY 576/589, BODIPY FL, BODIPY TMR, BODIPY TR, Carboxytetramethylrhodamine, Cascade Blue, a Coumarin, Cy2, CY3, CY5, CY9, Dansyl Chloride, DAPI, Eosin, Erythrosin, Ethidium Homodimer II, Ethidium Bromide, Fluorescamine, Fluorescein, FTC, GFP (yellow shifted mutants T203Y, T203F, S65G/S72A), Hoechst 33242, Hoechst 33258, IAEDANS, an Indopyras Dye, a Lanthanide Chelate, a Lanthanide Cryptate, Lissamine Rhodamine, Lucifer Yellow, Maleimide, MANT, MQAE, NBD, Oregon Green 488, Oregon Green 514, Oregon Green 500, Phycoerythrin, a Porphyrin, Propidium Iodide, Pyrene, Pyrene Butyrate, Pyrene Maleimide, Pyridyloxazole, Rhodamine 123, Rhodamine 6G, Rhodamine Green, SPQ, Texas Red, TMRM, TOTO-1, TRITC, YOYO-1, vitamin B12, flavin-adenine dinucleotide, and nicotinamide-adenine dinucleotide.

Claim 13 (currently amended): The method of claim 1 wherein said fluorochrome concentration is 1 nM or less and the measured fluorescence polarization of said mixture millipolarization is increased or decreased by at least 10 mP.

Claim 14 (original): The method of claim 1 wherein the said antibody is polyclonal or monoclonal.

Claim 15 (currently amended): The method of claim 8, wherein said bodily fluids are selected

from the group consisting of saliva, oral rinse expectorant, oral fluid including oral mucosal

transudate and gingival crevicular fluid, urine, sweat, tears, blood, serum, stool, gastric fluid,

synovial fluid, and phlegm, and other clinical and laboratory specimens and samples.

Claim 16 (withdrawn): A competitive method for estimating the concentration of specific antibody

in a sample of bodily fluids to a protein from Bacillus anthracis selected from the group consisting

of protective antigen or PA, lethal factor or LF and edema factor or EF said competitive method

selected from the group consisting of fluorescence polarization or FP, fluorescence lifetime or FLT

and fluorescence resonance energy transfer or FRET the said competitive method of claim

comprising the steps:

a. intermixing a said sample suspected of containing said antibody with a competitive

reagent labeled with a fluorochrome capable of binding to said specific antibody to

produce an antibody detection mixture;

b. incubating said antibody detection mixture for 15 seconds to 5 minutes;

c. detecting binding interaction between said protein and antibody.

Claim 17 (withdrawn): The method of claim 16, wherein said detection is by change in

fluorescence polarization.

Claim 18 (withdrawn): The method of claim 16, wherein said detection is by change in

fluorescence life-time.

5

Claim 19 (withdrawn): The method of claim 16, wherein said detection is by sensitized fluorescence of the acceptor or by quenching of donor fluorescence or by fluorescence depolarization.

Claim 20 (withdrawn): The method of claim 16, wherein said method comprises the additional steps of:

d. measuring the fluorescence polarization of a negative control solution of said fluorochrome-labeled competitive reagent, a positive control solution of said fluorochromelabeled competitive reagent exposed to a known amount of said antibody, or both, and e. comparing the measured fluorescence polarization of said mixture or said antibody detection mixture with the measured fluorescence polarization of said negative control solution, said positive control solution, or both.

Claim 21 (withdrawn): The method of claim 16, wherein said incubation step (b) occurs in less than 30 seconds for concentrated samples.

Claim 22 (withdrawn): The method of claim 16 wherein said incubation step (b) occurs in 4 to 5 minutes for low concentration samples.

Claim 23 (withdrawn): The method of claim 16 wherein said fluorochrome is pH independent.

Claim 24 (withdrawn): The method of claim 16 wherein said fluorochrome is selected from the group consisting of 7-AAD, Acridine Orange, Alexa 488, Alexa 532, Alexa 546, Alexa 568, Alexa 594, Aminonapthalene, Benzoxadiazole, BODIPY 493/504, BODIPY 505/515, BODIPY 576/589,

BODIPY FL, BODIPY TMR, BODIPY TR, Carboxytetramethylrhodamine, Cascade Blue, a
Coumarin, Cy2, CY3, CY5, CY9, Dansyl Chloride, DAPI, Eosin, Erythrosin, Ethidium
Homodimer II, Ethidium Bromide, Fluorescamine, Fluorescein, FTC, GFP (yellow shifted mutants
T203Y, T203F, S65G/S72A), Hoechst 33242, Hoechst 33258, IAEDANS, an Indopyras Dye, a
Lanthanide Chelate, a Lanthanide Cryptate, Lissamine Rhodamine, Lucifer Yellow, Maleimide,
MANT, MQAE, NBD, Oregon Green 488, Oregon Green 514, Oregon Green 500, Phycoerythrin,
a Porphyrin, Propidium Iodide, Pyrene, Pyrene Butyrate, Pyrene Maleimide, Pyridyloxazole,

Claim 25 (withdrawn): The method of claim 16 wherein said fluorochrome concentration is 1 nM or less and the sample millipolarization is increased or decreased by at least 10 mP.

Rhodamine 123, Rhodamine 6G, Rhodamine Green, SPQ, Texas Red, TMRM, TOTO-1, TRITC, YOYO-1, vitamin B12. flavin-adenine dinucleotide, and nicotinamide-adenine dinucleotide.

Claim 26 (withdrawn): The method of claim 16, wherein specificity of detection is 96 - 99%.

Claim 27 (withdrawn): The method of claim 16, wherein said bodily fluids are selected from the group consisting of saliva, oral rinse expectorant, oral fluid including oral mucosal transudate and gingival crevicular fluid, urine, sweat, tears, blood, serum, stool, gastric fluid, synovial fluid, phlegm, and other clinical and laboratory specimens and samples.

Claim 28 (canceled).